Docket No.: 0230-0245PUS1
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Hidemi KURIHARA et al.

Application No.: 10/571,069 | Confirmation No.: 2459

Filed: December 7, 2006 | Art Unit: 1649

For: THERAPEUTIC AGENT AND THERAPEUTIC METHOD FOR PERIODONTAL DISEASES AND PULPAL DISEASES | Examiner: C. M. Borgeest

DECLARATION SUBMITTED UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Hidemi KURIHARA of the Department of Periodontal Medicine, Division of Frontelet Medical Science, Graduate School of Biomedical Sciences, Hiroshima University, Japan, do hereby declare the following:

I am Professor and chair of Department of Periodontal Medicine and have worked in this field for 30 years.

I am one of the inventors of the above referenced patent application.

I am familiar with the patent application, as well as the development, usages and properties of polymer compounds.

I have read and understand the subject matter of the Office Action of April 5, 2010.

The following comments are offered in further support of the patentability of the instant invention.

Introduction

The Examiner has rejected claims 10-13, 15, 16 and 30 as obvious over Kirker-Head in view of Wikesjö, Tsuboi *et al.*, Kurihara *et al.* and Harada *et al.*

This declaration provides evidence demonstrating that the combination of BDNF and hyaluronic acid provided a significantly improved cementum generation as compared to the use of HMW-HA alone or BDNF with poly(lactic acid-co-glycolic acid)(PLGA).

An *in vivo* model of periodontal disease, to examine the effects of a BDNF/HMW-HA complex on periodontal tissue regeneration in inflamed class III defects in beagles was used to evaluate the effectiveness of the claimed processes.

A furcation defect is a term used to describe bone loss from periodontal disease, affecting the base of the root trunk of a tooth where two or more roots meet, i.e., the extension of periodontal pockets that may occur between the roots of multirooted teeth. In the study, the beagles were given a class III furcation defect, *i.e.*, a furcation defect encompassing the entire width of the tooth so that no bone is attached to the angle of furcation.

Two weeks after the surgical operation to simulate periodontal disease, BDNF/HMW-HA complexes having varying concentrations of BDNF were administered at the site of the furcation.

Complete regeneration of the periodontal tissues lost as a consequence of periodontal disease (or as artificially induced) requires the production of functional periodontal tissue composed of newly formed cementum and alveolar bone and growth of connective tissue fibers into these hard tissues; *i.e.*, regrowth of the periodontal ligament. The formation of cementum is a key phenomenon for establishing a functional periodontium.

BDNF in conjunction with HMW-HA unexpectedly provides significantly increased bone and cementum length when compared to either other type of complexes or to the HMW-HA complex lacking BDNF.

Materials and Methods

BDNF and synthesized high molecular weight hyaluronic acid (HMW-HA)

Recombinant human BDNF was supplied from Dainippon Sumitomo Pharmaceutical Co., Ltd. (Osaka, Japan). It was diluted with 10 mM sodium phosphate and 150 mM NaCl to 23.8 mg/ml (pH 7.0). The synthesized HMW-HA, the molecular weight of which was 2 million, was supplied from DENKA Co., Ltd. (Tokyo, Japan).

Experimental model for periodontal tissue regeneration

After having obtained the approval of the Committee of Research Facilities for Laboratory Animal Science of Hiroshima University School of Medicine, 10 female beagles (2 weeks observation model: 1 dog, 6 weeks observation model: 9 dogs) weighing 10-14 kg and aged 12-20 months were used in the present study. Their good oral health was ensured by scaling and mechanical tooth brushing.

Creation of class III furcation defects and transplantation of BDNF/HMW-HA complex

All surgical procedures were performed under general anesthesia induced with Nembutal® (40 mg/kg, Abbott Laboratories, North Chicago, IL) and local anesthesia induced with Xylocaine® (2% lidocaine with 1:80,000 noradrenaline, Fujisawa, Osaka, Japan). The mandibular second, third, and fourth premolars (P2, P3, and P4) on the right and left sides from 10 beagle dogs were used for the following experiment. After sulcular incisions had been made, full thickness flaps were raised, and class III furcation defects were surgically created (Fig. XA) with the use of bone chisels and slowly rotating round burs. Sterile saline was used to irrigate the soft and hard tissues during the surgical procedure. The defect height from the cement-enamel junction to the reduced alveolar crest was 4 mm. The exposed periodontal ligaments and cementum were completely removed to produce denuded root surfaces. Reference notches were placed on the mesial and distal roots to indicate the bottom of the defect. Then, the defects were filled with alginate impression material (MORITA, Tokyo, JAPAN) in order to induce inflammation (Fig. XB). The flaps were then replaced to their original position and sutured with interdental sutures.

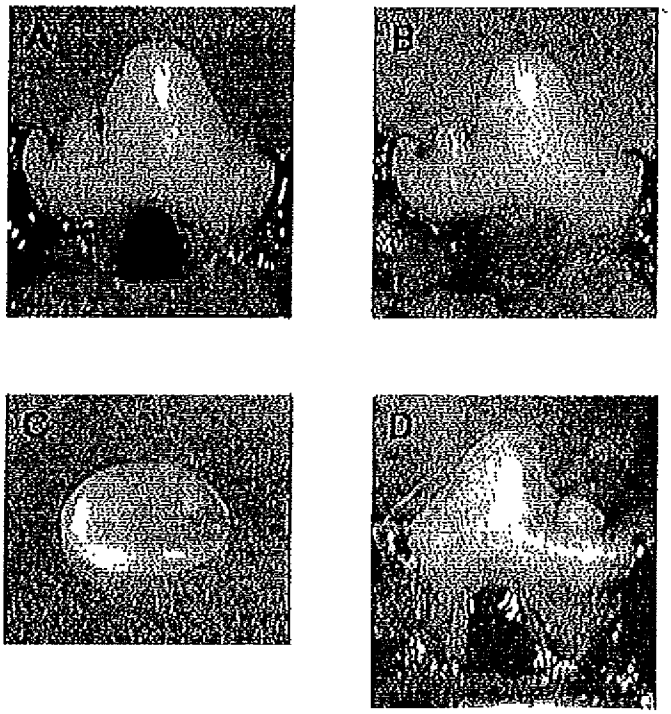
One week after the impression material had been inserted, it was surgically removed. During the next week, all test and control sites were mechanically cleaned by supra- and subgingival scaling and root planing for the prevention of plaque accumulation and periodontal inflammation.

One week after the removal of the alginate impression material, the reconstructive procedure was carried out. BDNF at concentrations of 5, 50, 500, and 2000 μg/ml was prepared by diluting it with HMW-HA (Fig. XC). After careful scaling and root planing, BDNF/HMW-HA complex was applied into the defects (Fig. XD). The flaps were then coronally repositioned

and sutured by the interrupted suture method with 4-0 silk sutures. During the subsequent 2 or 6 weeks, good oral hygiene was maintained by brushing and swabbing with 0.2% povidone iodine solution (Meiji-seika Co., Ltd., Tokyo, JAPAN). We compared the periodontal tissue regeneration in the BDNF/HMW-HA group with that in the HMW-HA alone application group and the sham operation group, which contains neither BDNF nor HMW-HA. We also compared the periodontal tissue regeneration in the BDNF/HMW-HA group with that in the BDNF (50 μg/ml)/PLGA (GC, Tokyo, Japan) group to examine the effectiveness of HMW-HA as a scaffold of BDNF.

Morphometrical analysis

Since 6 premolars were not available because of technical failure, 48 teeth from 9 dogs were used for the analysis of eight groups (sham operation, HMW-HA alone, 5 μg/ml BDNF+HMW-HA, 50 μg/ml BDNF+HMW-HA, 500 μg/ml BDNF+HMW-HA, 2000 μg/ml BDNF+HMW-HA, PLGA alone and 50 μg/ml BDNF/PLGA). The length of newly formed cementum and the area of newly formed bone were measured using the software NIH Image® on digitized photomicrographs captured by a Windows® computer. New cementum formation was represented as the percentage of new cementum length formed along the denuded root surface to the total root surface length from notch to notch (Fig. XE). The area of newly formed bone on each specimen was calculated as the percentage of the area surrounded by reference notches on the mesial and distal root surfaces facing the bone defect (Fig. XE). Since the periodontal ligament space is present in normal periodontal tissue, the percentage of bone area in normal specimens was 83%.



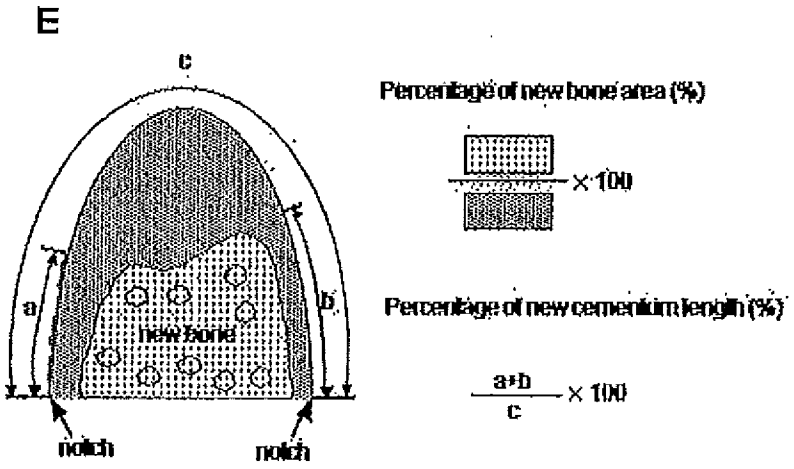


FIG. X. Creation of inflamed class III furcation defects and the application of BDNF. (A) Defect preparation; (B) Inserting the alginate impression material to induce inflammation; (C) Synthesized HMW-HA; (D) Application of BDNF/HMW-HA complex; (E) Schematic drawing of the histometric analysis of percentages of the new bone area and cementum length.

Results

Figure Y (below, previously submitted as Figure 1) demonstrates that the BDNF/HMW-HA complex provided significantly increased new bone area and cementum length. Morphometrical analyses of new bone area showed that the percentages of new bone area in the 5 μg/ml, 50 μg/ml, 500 μg/ml, and 2000 μg/ml BDNF+HMW-HA groups were 58.0±19.0, 67.7±12.5, 56.1±15.4, and 64.2±5.3, respectively (Fig. 6A). BDNF+HMW-HA at 50 and 2000 μg/ml significantly increased bone area compared to the HMW-HA group (Fig. 6A). Furthermore, the percentages of new cementum length in the 5 μg/ml, 50 μg/ml, 500 μg/ml, and 2000 μg/ml BDNF+HMW-HA groups were 75.1±16.5, 89.6±18.3, 77.8±10.1, and 79.8±12.4, respectively (Fig. 6B). BDNF+HMW-HA at 5, 50, and 2000 μg/ml significantly increased cementum length compared to the HMW-HA group (Fig. 6B).

Figure Y (aka Figure 1 as previously presented)

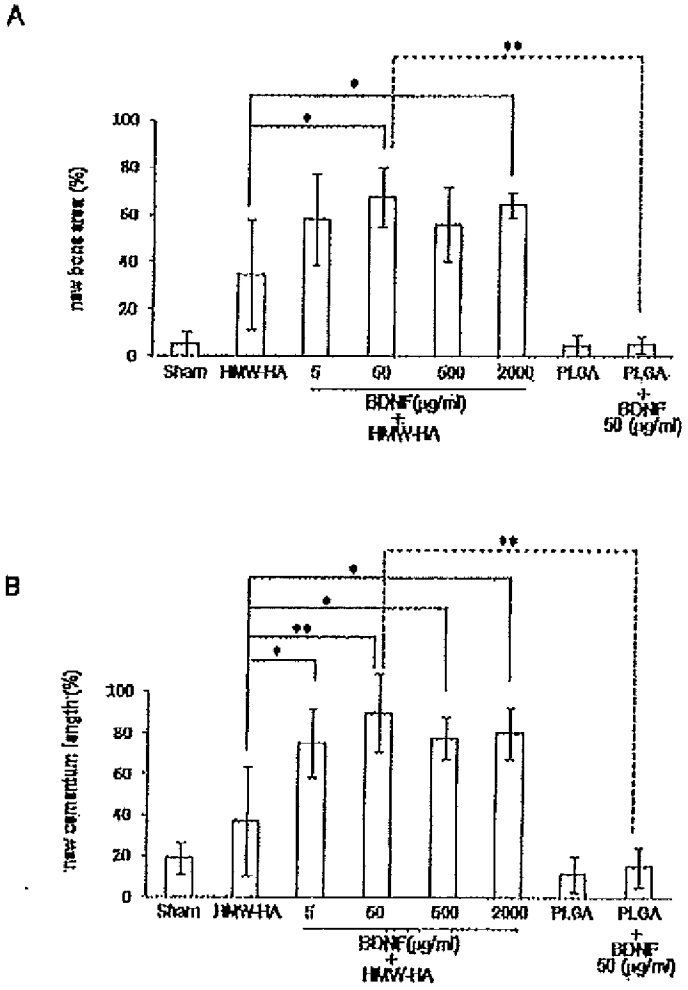


Fig. Y Morphometrical analysis of the effect of BDNF/HMW-HA complex. The graphs show the percentages of new bone area (A) and new cementum length (B) according to morphometrical analysis. Seven teeth from 9 dogs were used for the HMW-HA group and each BDNF/HMA-HA complex group. Three teeth from 9 dogs were used for the sham operation. Five teeth from 9 dogs were used for the PLGA group and the BDNF (50 μg/ml)/PLGA group. The tissues were decalcificated with KCX® (FALMA Co., Ltd) for 2 weeks. Three sections per tooth were examined for morphometrical analysis. The results of the HMW-HA group and the BDNF/HMW-HA groups are expressed as the mean ± S.D. of twenty-one sections for each group. The result of the sham operation group is expressed as the mean ± S.D. of nine sections. The results of the PLGA group and the BDNF (50 μg/ml)/PLGA group are expressed as the mean ± S.D. of fifteen sections for each group. Differs significantly (*P<0.05; **P<0.01) from the control.

Conclusion

Based on the data presented above with regard to Figure Y, it is clear that the presence of both BDNF and HA causes the significantly increased bone area and increased cementum length. In contrast, neither the BDNF on PLGA or HMW-HA carrier alone caused a significant increase in bone area or cementum length. In short, neither BDNF nor HMW-HA alone is responsible for the significant improvement in periodontal regeneration. Moreover, it is clear that using a different combination of BDNF and carrier does not result in the significant increase in bone area or cementum growth. Therefore, one of skill in the art would have found the significant increase in regeneration of periodontal tissue to be unexpected.

The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: Nov. 30, 2010 Hidemi Kurihara

Dr. Hidemi KURIHARA